Design of Novel Hsp90 Inhibitors to Trigger Tau Degradation in Alzheimer's disease: An Insilico Approach

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By

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CERTIFICATE

This is to certify that the thesis entitled **"Design of Novel Hsp90 Inhibitors to Trigger Tau Degradation in Alzheimer's disease: An Insilico Approach"** submitted by **Mr SHAIK KAZAVALI** in partial fulfilment of the requirements for the award of bachelor of Technology in Biotechnology and Medical engineering with specialization in Biotechnology at the National Institute of Technology, Rourkela is an genuine work carried out by him under my supervision and guidance.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any Degree or Diploma.

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CONTENTS

Abstract

Alzheimer's disease (AD) is one of the most common Protein amyloid-associated Neurodegenerative disorder which is an irreversible and progressive patterns of cognitive and functional impairments. The neuropathological identifications and findings of AD includes presence of plaques and neurofibrillary tangles.Tau hypothesis is a model of neurofibrillary tangles where, hyper phosphorylated tau begins to misfold with other threads of tau. Ultimately, they lead to form neurofibrillary tangles in nerve cells. This involves in the disruption of microtubules, and eventually leads to breaking down of neuron's passage coordination. This may result in a glitch of biochemical intercommunication between neurons and subsequently leads to death of neurons. Hsp90 is a highly protective molecular chaperone and its functions are stress responses, aids other proteins to fold perfectly, stabilities proteins against heat stress, and helps in protein degradation, and assembly of other vast chaperones for numerous biological purposes. Tau which is a client protein of Hsp90 has been reported to be an important cause of AD. Inhibition of Hsp90, hence has been shown to be a strategy of therapy by directing tau towards degradation pathway. In the present investigation, we have designed novel analogues of geldanamycin, novobiocin as Hsp90 inhibitors and assessed their inhibitory potential. We concluded that analogues-3 of geldanamycin was found to be a better Hsp90-inhibitor than geldanamycin.

Key words: Hsp90, Hsp90 inhibitors, tau aggregation, Alzheimer's disease, Insilico design, Novobiocin, Geldanamycin, Docking.

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Chapter 1

INTRODUCTION

1.1 Introduction:

Alzheimer's disease was mainly due to β-Amyloid and its related condition, inflexible, escalating brain disease that results in nerve cells disintegrating the thinking, memory, remembering, reasoning and skills of individuals [1]. The neuropathological identifications and findings of AD incorporate vicinity of plaques and neurofibrillary tangles.Tau hypothesis was a model of neurofibrillary tangles where, hyper phosphorylated tau begins to misfold with other threads of tau. Ultimately, they lead to form neurofibrillary tangles in nerve cells [1]. This involves in the disruption of microtubules, and eventually leads to breaking down of neuron's passage coordination. This results in a glitch of biochemical intercommunication between neurons and subsequently leads to death of neurons. Validation of tau hypothesis also confirms the actuality of other diseases called as tauopathies, which refers to same protein which was identified as misfolded. Although, maximum researchers proved another hypothesis that was beta-amyloid the ultimate causing agent [1]. Tau recognized by the chaperone system is processed with Hsp70/Hsp40 complex and then forms an intermediate complex with Hsp90. Hsp90 inhibitors then promote tau degradation [1].

1.1.1 Neurodegenerative disorders and Alzheimer's Disease :

Neurodegeneration was an aegis term for the continuous loss of structure or purpose of nerve cells, which including disintegration of nerve cells. Most commonly seen neurodegenerative disorders comprise of ALS, Parkinson's, Alzheimer's, and Huntington's disorders [1, 2].

AD was identify by loss of nerve cells and Synaptic cells in the cerebral cortex and subcortical dementia regions. AD was mainly due to Beta-Amyloid and its related condition, inflexible, escalating brain disease that results in nerve cells disintegrating the thinking, memory, remembering, reasoning and skills of individuals. The neuropathological identifications and findings of AD incorporate vicinity of senile plaques and neurofibrillary tangles [2].

Tau hypothesis: Tau hypothesis was a model of neurofibrillary tangles where, hyper phosphorylated tau begins to misfold with other threads of tau. Ultimately, they lead to form neurofibrillary tangles in nerve cell. This involves in the disruption of microtubules, and eventually leads to breaking down of Neuron's passage coordination. This results in a glitch of biochemical intercommunication between neurons and subsequently leads to death of neurons and results in neurodegeneration [2].

Symptoms: This illness course is partitioned into four stages, with dynamic patterns of cognitive and functional impairments.

1.1.2 Heat shock protein 90(Hsp90):

Hsp90 was a highly protective molecular chaperone and its functions are stress responses, aids other proteins to fold perfectly, stabilities proteins against heat stress, and aids in protein degradation, and assembly of other vast chaperones for numerous biological purposes. Inhibition of tau degradation by Hsp90 has been investigated to be a medicinal diagnosis of Alzheimer's disease. Hsp90 chaperone also shield the mutated Tau-proteins from misfolding and lead to proteasomal disintegration. That validated that Hsp90 ATPase task is up regulated nearly 100 times in Tau aggregation. Also, the proper assumption might be the up regulation of its activity as a chaperone [2, 3].

Hsp90 is a part of superior family that constitutes an ATP binding site which was unalike other ATP binding pocket of protein kinases. The developmental conserve of this chaperone structure contains of three domains. Those domains were composed of almost 732 amino acids. Hsp90 also has two isomers α and β, mainly found in cytosol. N –terminal domain was an amino terminal domain which consists a fold called as bergerat fold which mainly constitutes ATP and inhibitor binding site. And the middle domain was having a cochaperone orientating motifs that gives molecular docking sites for target proteins and co-chaperones plays a counterpart in making the excited ATPase. Finally, C-terminal domain is the carboxyl terminal domain which was having dimerization motif that was a second drug orientation location and pocket of communicating with other co-chaperones molecules in the body. Dimerization of Hsp90 monomer via C-terminus is Crucial for chaperoning function [2, 3].

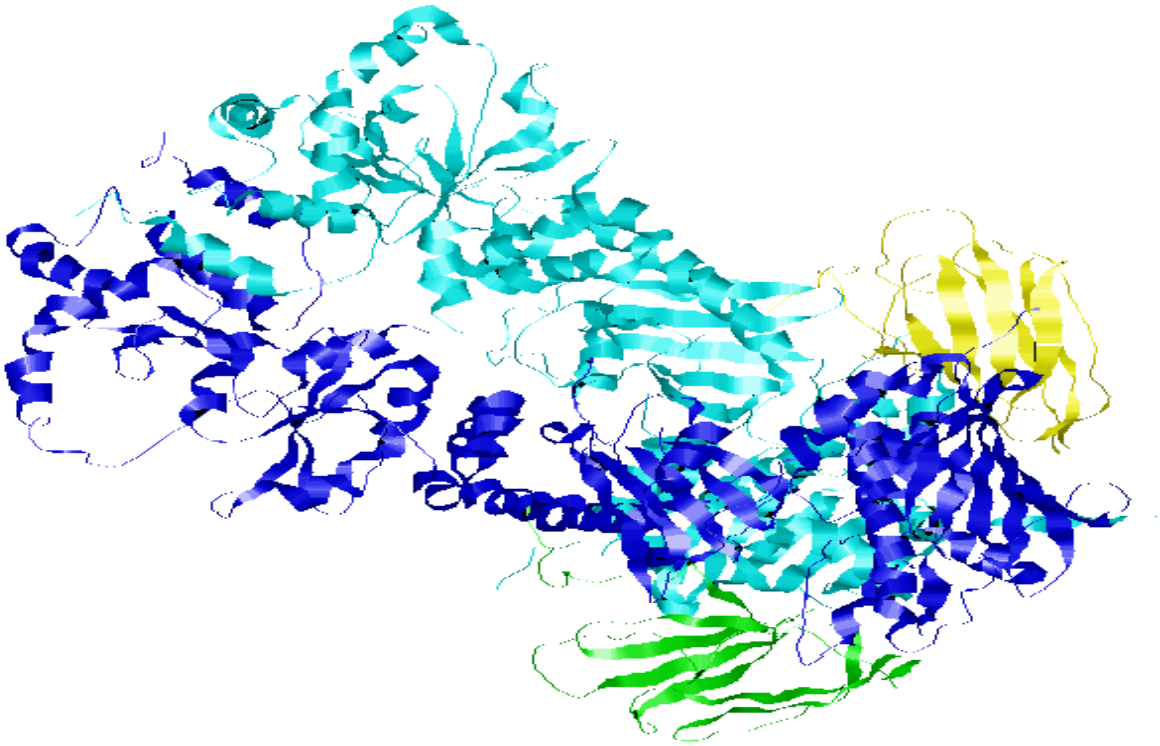

Figure 1. Structure of Hsp90.

1.1.3 Info Regarding Bioinformatics and Computational Biology:

Mainly, Computational biology and Bioinformatics have the capacity to increase speed of inhibitor analysing methods, decreasing the expenses of the methods and ultimately modify the drugs which were designed [7]. Rational drug design eases and increases the speed of drug modification methods which contains numerous technique results in making novel molecules. Most improving technique was the molecular docking by which ligand or inhibitor with the target sites. Locations where the inhibitor interacts were called as the active site, and those were reasonable with the pharmaceutical results with the inhibitor [7]. Molecular docking is the process in which two molecules interact with one another in a 3D space. In inclusion with, retrogression located or recognition based point scoring functions which can be useful to calculate the free enthalpy of inhibition interaction. Numerous tools, software and servers were used though out world for molecular docking computation. There were fixed, adjustable, and semi adjustable docking [7]. Many different databases stores macromolecular 3D structure of proteins and inhibitors structure, which were obtained from various NMR co-ordinates were used for docking and simulations. Ultimately computational work or Insilico methods were maturing every day with many improvements. And becoming a reliable field where the time and cost of computational methods related to inhibitory study, molecular orientations were decreasing [7].

1.2 Objective:

- Retrieval of amino acid sequence of human Hsp90 protein from NCBI.
- Modelling of Hsp90 Protein using PHYRE 2 server.
- Designing of novel Geldanamycin and Novobiocin analogues Insilico.
- Estimation of binding affinity of newly designed analogues for Hsp90.
- Finding the best designed Hsp9-inhibitor in Alzheimer disease.

Chapter 2

LITERATURE REVIEW

2.1 Alzheimer's disease as a Neurodegenerative Disorder:

Neurodegeneration is an aegis term for the continuous loss of structure or purpose of nerve cells, which including disintegration of nerve cells. Most commonly seen as neurodegenerative disorders comprise of ALS, Parkinson's, Alzheimer's, and Huntington's disorders. AD was mainly due to β-Amyloid and its related condition, inflexible, escalating brain disease that results in nerve cells disintegrating the thinking, memory, remembering, reasoning and skills of individuals [4]. The neuropathological identifications and findings of AD incorporate vicinity of senile plaques and neurofibrillary tangles. Tau hypothesis is a model of neurofibrillary tangles where, hyper phosphorylated tau begins to misfold with other threads of tau [4]. Ultimately, they lead to form neurofibrillary tangles in nerve cells. This involves in the disruption of microtubules, and eventually leads to breaking down of neuron's passage coordination. This results in a glitch of biochemical intercommunication between neurons and subsequently leads to death of neurons [4]. AD is identify by loss of nerve cells and Synaptic cells in the cerebral cortex and subcortical dementia regions. AD is mainly due to β-Amyloid and its related condition, inflexible, escalating brain disease that results in nerve cells disintegrating the thinking, memory, remembering, reasoning and skills of individuals. The neuropathological identifications and findings of AD incorporate vicinity of senile plaques and neurofibrillary tangles [4, 5].

This AD was coined Dr. Alois Alzheimer. Dr. Alois Alzheimer identified this modification in the brain tissue of various persons how would die due of mental disorder. Plaques and tangles are identified as the malfunction of nerve system and destroys the communication within the nerve cells because the nerve cells could not connect within them and results in death due to AD. Removal of unusual form of plaques and tau tangles [5].

2.1.1 Symptoms of Alzheimer's diseases:

Alzheimer's disease leads to certain symptoms that involves mental malfunction, including:

- Memory failure,
- Anger
- cogitate problems
- Distressing behaviour.
- Loss of rationality

- Analytical distress
- Memory loss.
- Low performance in complicated activities.
- Changing patterns of sleep and wakefulness in nights.
- Getting illusions, lethargic.
- Loss of responsibilities.
- Loss of ability like reading and writing.
- Mal functionality in the ability for recognize danger.
- Incorrectness of statement, pronouncing mistakes.
- Isolating himself from social environment.
- Talking ability
- Identification of relatives

Most of the difficulty that people have regarding this disease are by faces unrestraint, problems in food intake, and lags in awareness and have difficulty in identifying the word. Warning signs of AD are [5]:

- Mystification of locations and presence.
- Analytical distress in problem solving.
- Memory loss of day-to-day activities.
- Unable to identify relative in his family.
- Isolation of environment.
- Difficulty in identifying objects.
- Lags in awareness.
- Characteristic behaviour changes.

2.1.2. Causes of Alzheimer's disease:

Alzheimer's disorder was identify by loss of nerve cells and Synaptic cells in the cerebral cortex and subcortical dementia regions. AD was mainly due to β-Amyloid and its related condition, inflexible, escalating brain disease that results in nerve cells disintegrating the thinking, memory, remembering, reasoning and skills of individuals. The neuropathological identifications and findings of AD incorporate vicinity of senile plaques and neurofibrillary tangles [6].

2.1.2.1 Genetic Disorder:

About 0.1% situations where parental related inactive autosomal taken by heritage, usually it is in the range of age 65. This disease is called as before outbreak of parental AD. All of inactive autosomal parental AD could be assigned to radiate with the following three genes: which encodes β-Amyloid and presenilins 1 and 2 [8]. Rest radiations in the β-Amyloid and presenilin genes allows the increase in preparation of a small protein known as Aβ42, and a main constituent of Amyloid plaques. Many of the radiations lowly change the ratio within Aβ42 and the rest of the other major forms e.g., Aβ40— lacking the increasing of Aβ42 content. Proposition of presenilin radiations results in disease causing and leads to lowering most of the amount of Aβ produced and results to other roles of presenilin. Almost every human disorder, ecological impacts and hereditary modifiers bring about fragmented penetrance. Radiations in the TREM2 gene results with a 3 to 5 time's increasing risk of acquiring Alzheimer's disease [8].

2.1.2.2 Cholinergic hypothesis:

One of the Ancient methods, by that all present done drug diagnosis were based, and it is the cholinergic hypothesis, that proves this AD is due to the reduction preparation of neurotransmitter acetylcholine. And this theory indicates cholinergic hypothesis that has not been maintained widespread to support, largely because the diagnosis which is involved to treat acetylcholine lack has not been very involving. Other cholinergic side-effects have also been discussed, for example, firstly formation of large-scale aggregation of β-amyloid, leading to common neuroinflammation.

2.1.2.3 Amyloid hypothesis:

In 1991, β-Amyloid and presenilin genes allows the increase in preparation of a small protein known as Aβ42, and a main constituent of Amyloid plaques. Many of the radiations lowly change the ratio within Aβ42 and the rest of the other major forms e.g., Aβ40— lacking the increasing of Aβ42 content. Proposition of presenilin radiations results in disease causing and leads to lowering most of the amount of Aβ produced and results to other roles of presenilin [6, 8]. Almost every human disorder, ecological impacts and hereditary modifiers bring about fragmented penetrance. Radiations in the TREM2 gene results with a 3 to 5 time's increasing risk of acquiring AD. In 2009, Ultimate theory was evolved, indicating the close relative of the beta-amyloid protein. And holds well that a β-amyloid-related system that increases neuron connections in the cortex and results in increasing -growth phase of all life of AD [8].

2.1.2.4 Tau hypothesis:

Tau hypothesis was a model of neurofibrillary tangles where, hyper phosphorylated tau begins to misfold with other threads of tau. Ultimately, they lead to form neurofibrillary tangles in nerve cells. This involves in the disruption of microtubules, and eventually leads to breaking down of neuron's passage coordination [9]. This results in a glitch of biochemical intercommunication between neurons and subsequently leads to death of neurons and results in neurodegeneration.

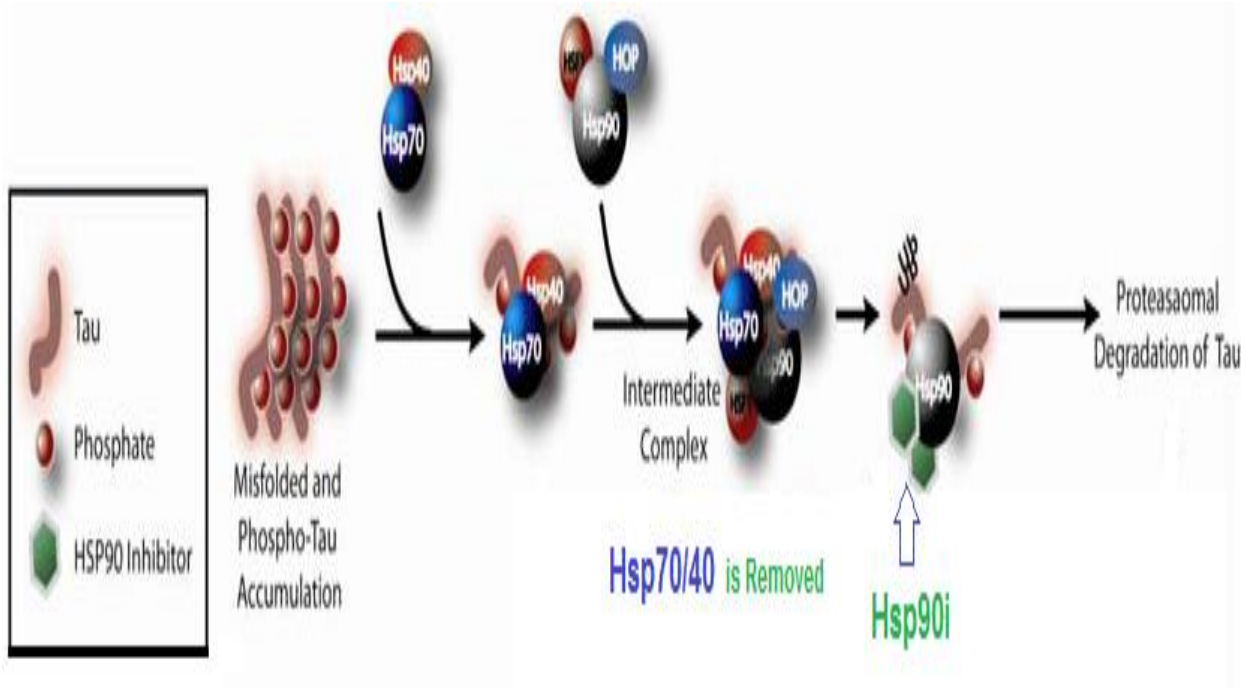

Figure 2. Visualization of HSp90 involvement in tau aggregation.

2.2 Heat shock proteins [also known as molecular chaperones]:

Hsp90 was a highly protective molecular chaperone and its functions are stress responses, aids other proteins to fold perfectly, stabilities proteins against heat stress, and helps in protein degradation, and assembly of other vast chaperones for numerous biological purposes. Inhibition of tau degradation by Hsp90 has been investigated to be a medicinal diagnosis of AD [9]. Hsp90 chaperone also shield the mutated Tau-proteins from misfolding and lead to proteasomal disintegration. That validated that Hsp90 ATPase task is up regulated nearly 100 times in Tau aggregation. Also, the proper assumption might be the up regulation of its activity as a chaperone.

- Hsp90 is a part of superior family that constitutes an ATP binding site which was unalike other ATP binding pocket of protein kinases. The developmental conserve of this chaperone structure contains of three domains. Those domains were composed of almost 732 amino acids. Hsp90 also has two isomers Alpha and Beta, mainly found in cytosol. N –terminal domain was an amino terminal domain which consists a fold called as bergerat fold which mainly constitutes ATP and inhibitor binding site [10].
- And the middle domain was having a cochaperone orientating motifs that gives molecular docking sites for target proteins and co-chaperones plays a counterpart in making the excited ATPase. Dimerization of Hsp90 monomer via C-terminus is Crucial for chaperoning function [9, 10].
- Small heat shock proteins (Hsp25) [Holder]
  - Protect against cellular stress.
  - Prevent aggregation in the lens (cataract).
- Hsp60 system ATPase [Unfolders]
  - Protein folding.
- Hsp70 system ATPase [Unfolders]
  - Stabilization of extended chains.
  - Membranes translocation.
  - Regulation of heat shock response\
- Hsp90 [Holder]
  - Binding and stabilization/regulation of steroid receptors, protein kinases.
  - Buffer for genetic variation.
- Hsp100 ATPase [unfolder]
  - Thermo tolerance, proteolysis, resolubilization, of aggregates.

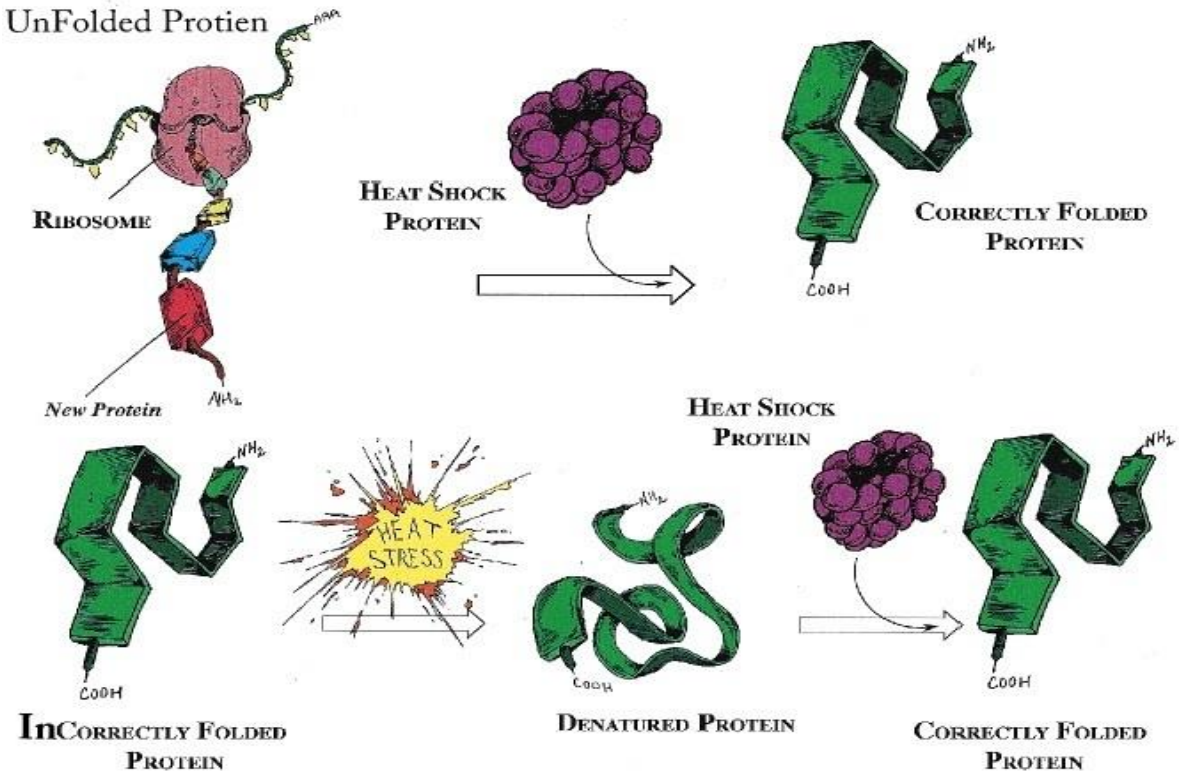

Figure 3. Main functions of a Heat shock Protein.

2.3 Heat Shock Protein 90KDa [Hsp90]:

It is a Chaperone protein which in association with the Co-chaperones aids different proteins to fold properly, stabilizes out proteins against high temperature stress, and supports in protein degradation. It's Molecular Wt. = 83.264KDa. Hsp90 is a cytosolic protein, nearly present 1-2% in the cytosol. Its Concentration varies depending on cells. It is over expressed in cancer cells and estimated to be 2.8% in colon cancer cells [9].

2.4 The structure of Hsp90:

The general structure of Hsp90 is like that of different proteins in that it holds the greater part of the basic secondary structural components. Being a cytoplasmic protein obliges that the protein be globular in structure, that is generally non-polar within and polar on the outside, in order to be dissolved by water. Hsp90 holds nine helices and eight against-parallel beta pleated sheets, which consolidate to structure a few alpha/beta sandwiches. The 310 helices make up more or less 11% of the protein's amino acid residues, which is much higher than the normal 4% in different proteins [10].

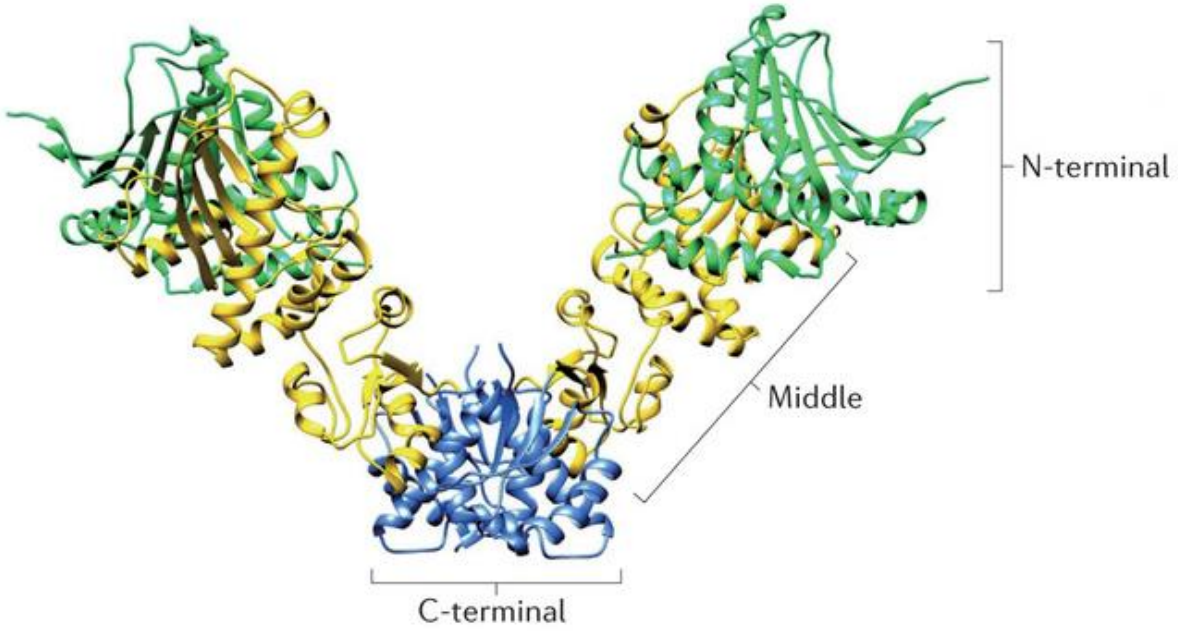

Figure 4. Crystal structure of Hsp90 [PDB ID-2IOQ].

2.4.1 The Structural Analysis of Hsp90:

In Eukaryotic cells, there are two Hsp90 isoforms [i.e. α, β] which are encoded by two separate genes. Hsp90α exists predominantly as a homodimer. Hsp90β basically as monomer. Examination by PAGE uncovered that bacterially expressed Hsp90α fused to GST. Limiting proteases of C-terminal 200 amino acids of Hsp90α with chymotrypsin produce C-terminal 16 KDa fragment [11].

[Met 628/Ala 629 - Asp 732]-This is the location of dimer site in an Hsp90 protein.

Its adjacent N-terminal 13KDa fragments

[Val 542 – Try627/Met 628]- This is the location os dimer site in an Hsp90 protein.

By analysing the Hsp90 [3Q6M] .pdb file of Hsp90 proteins C-terminal binding domains was analysed using CASTp online server to find the active site of the protein. Thus, the site residue of protein were found and the active part involved in dimerization is opted for further docking with the inhibitors for stopping tau aggregation [10, 11].

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2.4.2 Hsp90 as a molecular chaperone:

In Eukaryotic cells, there are two Hsp90 isoforms [i.e. α, β] which are encoded by two different genes. Hsp90α exists overwhelmingly as a homo .Hsp90β principally as monomer. Observation by PAGE uncovered that bacterially expressed Hsp90α combined to GST [12]. The molecular chaperone heat shock protein 90 [hsp90] is an essential and abundant protein in eukaryotic cells, vital for actuation of a vast set of signal transduction and administrative protein. Throughout the functional cycles, the Hsp90 performs extensive conformational adjustment. The transient N-terminal dimerization of Hsp90 has been broadly examination, under the supposition that C-terminal interface is stable dimer [12].

By analysing Hsp90 .pdb file for [C-terminal and binding] domain, thus analysis using CASTp software to find active residue of the protein. The active residue were found and active parts involved in dimerization were opted for further docking with the inhibitors for stopping of Tau aggregation [12, 13].

2.5 The Functions of Hsp90 in various biological processes:

Hsp90 can efficiently bind to the target proteins and is crucial for their folding, maturation and maintaining in the folding competent. Members of Hsp90 bind to various peptides in vivo and in vitro. Hsp90 involvement in signalling processes, poses threat to cellular function. Extracellular Hsp90 interacted with the receptor CD91 [14]. Tau recognized by the chaperone system is processed initially by the Hsp70/Hsp40 complex and then forms an intermediate complex with Hsp90 and HOP. Hsp90 inhibitors can promote tau degradation.

Hsp90 assumes clearly clashing roles in the cell, as it is fundamental for both the creation and the support and additionally the annihilation of proteins. Its normal function is basic to keeping up the health of cells, though its dysregulation may help carcinogenesis [15]. The capability of this chaperone to both balance out the 26s proteasome (which empowers the cell to degrade unwanted and/or destructive proteins) and to stabilize kinases against the same proteasome exhibits its useful differences. The uses of Hsp90 inhibitors in disease medication highlight Hsp90's significance as a therapeutic target [14, 15]. Hsp90 beta has been identified as one of the auto antigenic biomarkers and targets included in human ovarian autoimmune ailment prompting ovarian mal function and in this way infertility [16].

2.6 Overexpression of Hsp90 in Alzheimer's disease:

Tau hypothesis was a model of neurofibrillary tangles where, hyper phosphorylated tau starts to misfold with different threads of tau. At last, they structure neurofibrillary tangles inside nerve cell bodies. This effects in the breaking down of microtubules, and in the long run prompts collapsing of neuron's transport framework. This may come about first in glitches in biochemical intercommunication between nerve cells and later in the death of nerve cells. Tau recognized by the chaperone system is processed initially by the Hsp70/Hsp40 complex and then forms an intermediate complex with Hsp90 and HOP. Hsp90 inhibitors can promote tau degradation [13, 16].

Hsp90 is known to connect with the non-local structures of numerous proteins, which has prompted the recommendation of the Hsp90 which included in protein folding as a rule. Eukaryotic proteins that are no more required or are misfolded or generally harmed are typically marked for destruction by the polyubiquitation pathway. These ubiquitinated proteins are perceived and degraded by the 26s proteasome. Subsequently the 26s proteasome is an indispensable some piece of the cell's system to degraded proteins. Besides a steady supply of useful Hsp90 is required to keep up the tertiary structure of the proteasome. At long last examinations done with heat sensitive Hsp90 mutants and the 26s proteasome recommend that Hsp90 is responsible for most, if not all, of the ATPase action of the proteasome [6, 7].

2.7 Hsp90 Inhibitors:

An Hsp90 inhibitor is a substance that hinders that movement of the Hsp90 heat shock protein. Since Hsp90 stabilizes a numerous variety of proteins needed for survival of disease cells, these substances may have therapeutic benefit in the medication of different sorts of malignancies. In current Hsp90 inhibitors are produced from geldanamycin and radicicol which are the regular product inhibitors and are beginning stage for new approach [17]. HSP 90 is needed for ATP dependant refolding of denatured or unfolded proteins and for the conformational development of a subset of proteins included in the reaction of cells to extracellular signs. It ties ATP & ADP and has feeble ATPase movement. This recommends that site demonstrations as nucleotide or nucleotide degree sensor. It is watched that nucleotides embrace special C molded twisted shape when trying to this pocket. This is especially unordinary as nucleotides never embrace shape change in high affinity ATP/ADP locales [17]. This likewise shows that drugs that are created ought to additionally can possibly receive special C shape adaptation conformation request to predicament the one of a special pocket.

The sane for this irregular need i.e. to twist the structure, is focused around thermo dynamical certainty that the particle which needs least structural progressions to go from unbound to bound state ought not to pay much entropic punishments and tying might be reflected by enthalpic components. Geldanamycin and Radicicol hardly ties to this pocket and keep the arrival of protein from chaperone complex. Accordingly the protein can't attain local conformation and is degraded by proteosome. It is worth to note that the ordinary partners are not hindered [18].

Geldanamycin is a successful Hsp90 inhibitor still it can't be utilized as a part of vivo in view of its high lethality and liver harm capacity. The theory is that the benzoquinone practical gathering is mindful. Hsp90 keeps the death proteins in an apoptosis safe state by immediate acquaintances. Its extensive variety of capacities effects from the capacity of Hsp90 to chaperone a few targeted proteins that assume a focal pathogenic part in human diseases including malignancy, neurodegenerative illnesses and viral contamination. Geldanamycin straightforwardly ties to the ATP-tying pocket in the N-terminal area of Hsp90 and, subsequently, obstructs the coupling of nucleotides to Hsp90 [18, 19].

Chapter 3

TOOLS AND METHODS

3.1 Bioinformatics Tools and software Used:

Computational science and Bioinformatics can possibly accelerate drug finding methods, diminishing the expenses of the procedures and changing the way the pills are composed. Normal drugs configuration encourages and accelerates the medication planning methods that includes different strategy for distinguishing novel compounds. One progressed technique is the docking of ligand or inhibitor with the target. The site where the inhibitors ties is known to the site of activity, which is answerable for the pharmaceutical impact in the target. Docking is the strategy by which two atoms tie to one another in 3d space. There are different apparatuses, programming and servers implied for docking computations.

3.1.1 Soft wares required:

- Swiss-PdbViewer 4.10
- Argus Lab 4.0.1
- Auto Dock 4.0
- Auto DockVina 1.0
- Chimera 1.6.1
- MGL Tools 1.5.6
- Open Babel 2.3.2
- Python 2.7.6
- ChemBioDraw 13.0

3.1.2 Online servers used:

- http://www.rcsb.org/
- https://pubchem.ncbi.nlm.nih.gov/
- http://www.msdiscovery.com/natprod.html
- http://www.swissdock.ch/
- http://projects.biotec.tu-dresden.de/metapocket/
- http://dogsite.zbh.uni-hamburg.de/
- http://sts-fw.bioengr.uic.edu/castp/calculation.php
- http://uniprot.org
- http://bioserver-3.bioacademy.gr/Bioserver/ChemBioServer/Toxic.php
- http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg

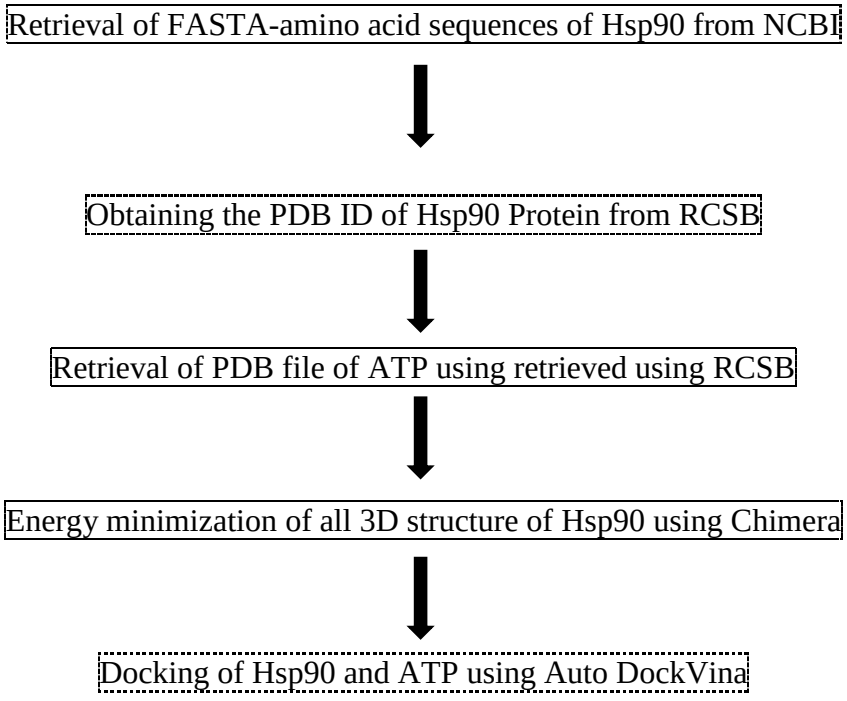
3.1.3 Files Required:

- PDB file of targeted protein[Hsp90]
- SDF files of Hsp90 Inhibitors
- PDB files of Hsp90 Inhibitors using ProDRG.
- PDBQT files of both targeted protein[Hsp90] and Its Inhibitors

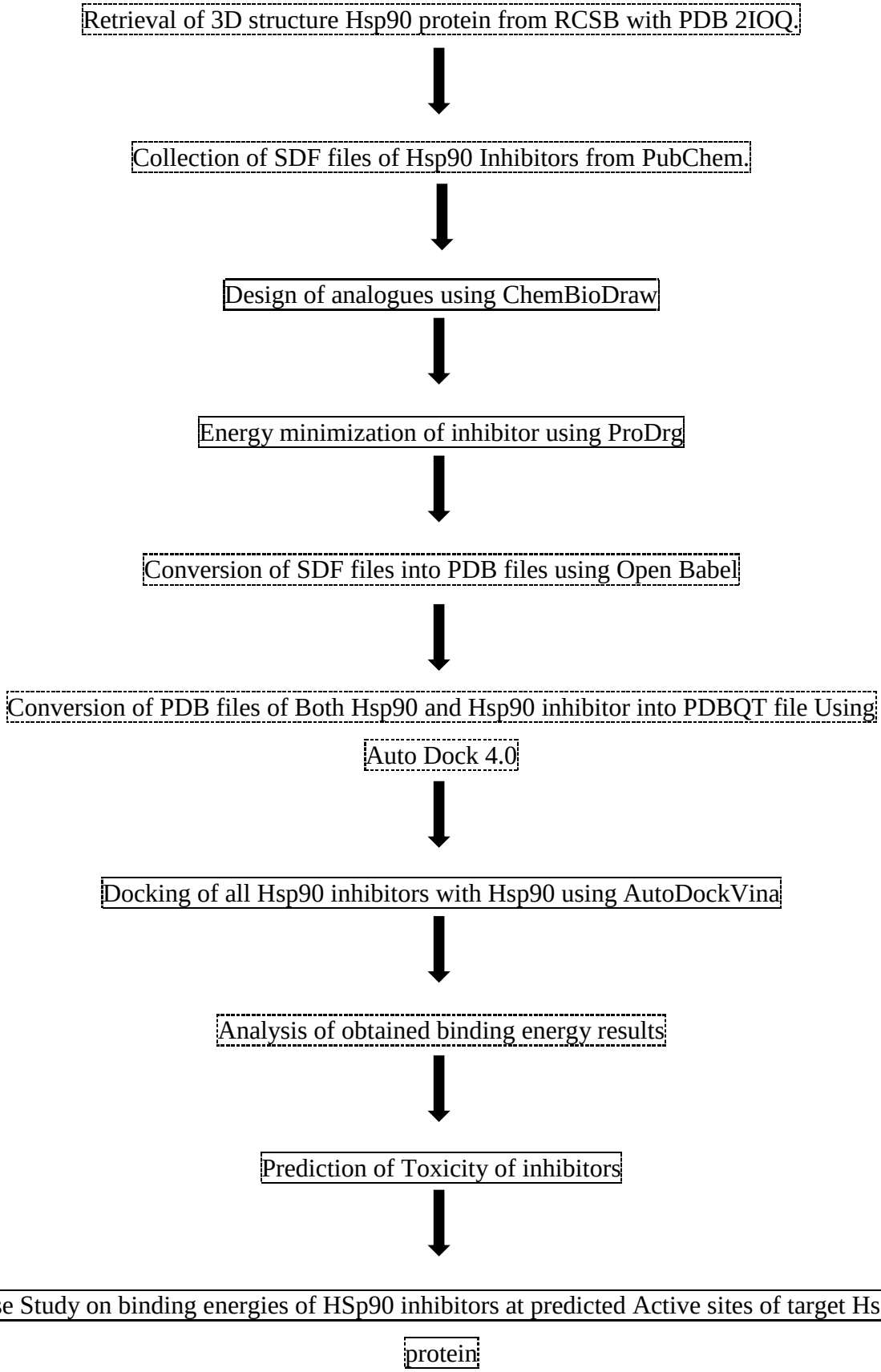
3.2 Protocol:

In this Insilco based screening, we have selected Hsp90 (PDBID-2IOQ) which plays a critical role in the development of Tau Aggregation which is responsible for one of the main cause of Alzheimer's disease.

3.2.1 Interaction between Hsp90 and ATP:



3.2.2 **Inhibition of Hsp90:**



3.3 Methodology:

Molecular docking is a method which is used for the prediction of preferred orientation of one molecule to other molecule when bound form a stable. It is frequently used to predict the binding energy of small molecular drugs to the targeted protein in order to predict affinity and activity.

3.3.1 Retrieval of FASTA-amino acid sequences of Hsp90 Protein using NCBI:

NCBI stands for National Centre for Biotechnological Information. It is created as a division of National Library of Medicines at National Institutes of Health. The NCBI answerable for making mechanized frameworks of information about molecular biology, natural chemistry, and genetics, giving the utilization of such databases and programming by the exploration and therapeutic group; gather biotechnology data both broadly and globally; and execution explore on praiseworthy routines for machine-based data handling for looking at the structure and capacity of naturally paramount molecules. The URL for this database is http://www.ncbi.nlm.nih.gov.

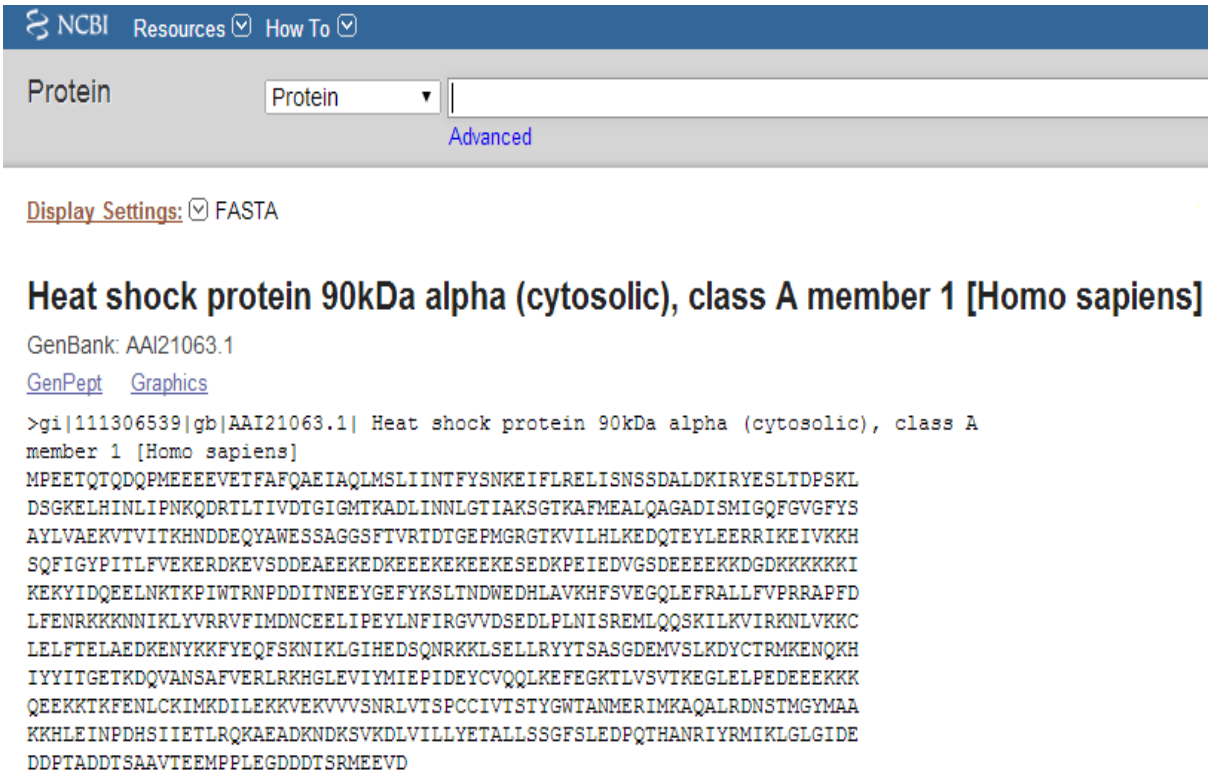


Figure 5. FASTA sequence of Hsp90 is retrieved from NCBI.

3.3.2 Retrieval of modelled 3D-structure of Hsp90 Protein using PHYRE 2 server:

PHYRE is an automatic fold recognition server for calculating the structure and function of the protein sequence that got submitted in the server. It is used for academic users only. It uses the principle and technique of Homology Modelling and relies on Hidden Markov Models.

Figure 6. Submission of Hsp90's amino acid sequences in PHYRE 2 server.

3.3.3 Retrieval of PDB file of ATP molecule using PubChem:

The PDB (Protein Data Bank) is the general store of Structural information of Biological Macromolecules, established in Brookhaven National Laboratories (BNL) in 1971.it gives Structural data of the macromolecules evaluated by X-beam crystallographic, NMR Methods. This is very much important as the understanding of shape will lead to the way how it functions. As biological macromolecule like protein is having a structure to function relationship. Hence an accurate knowledge of structure is needed to know the varying functions. This server is free of use.one can easily download the structure in pdb file format or Fasta format.

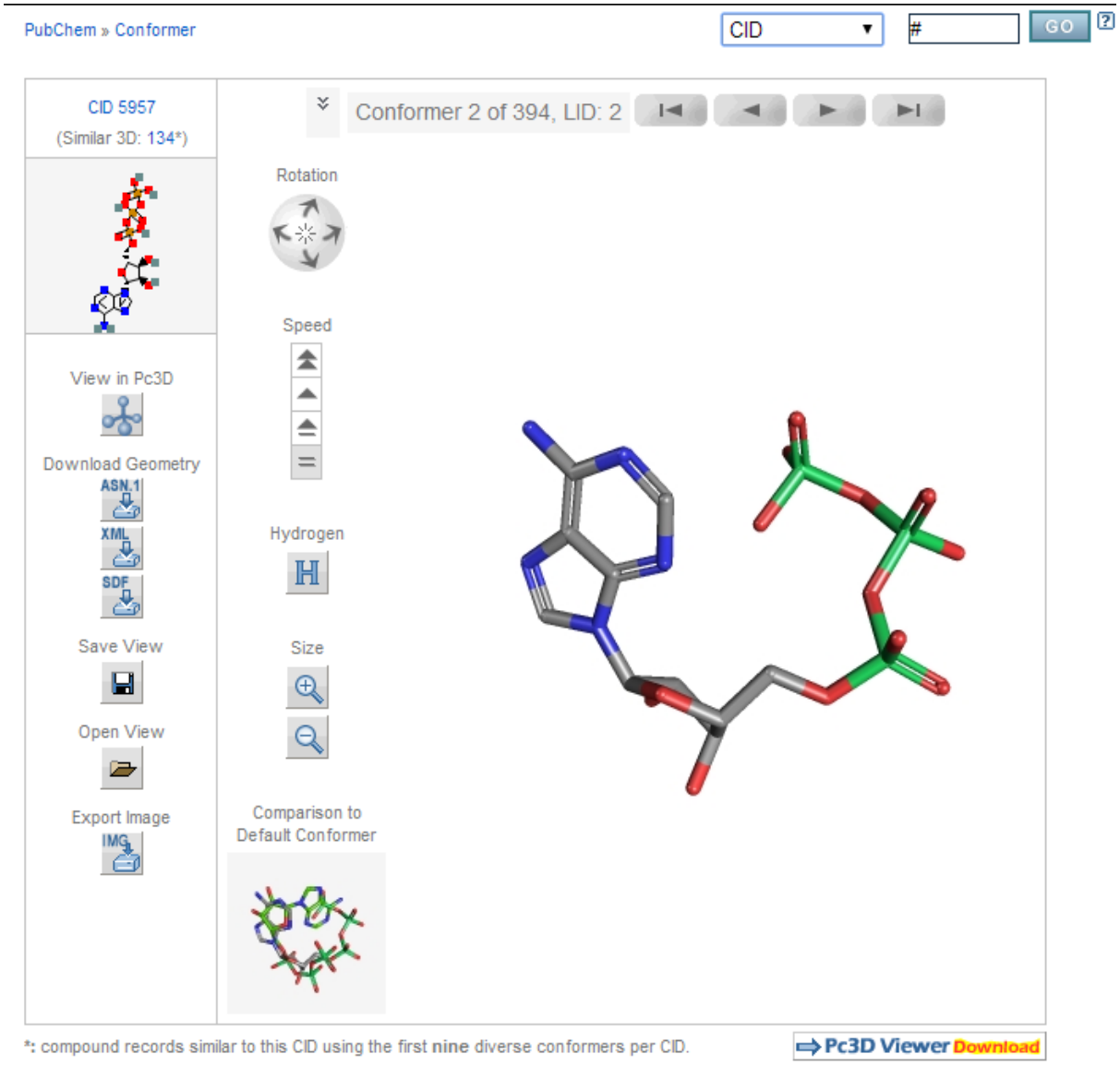


Figure 7. Visualization of ATP molecule using PubChem.

3.3.4 Energy minimization of Hsp 90 molecule using Chimera 1.7:

UCSF CHIMERA 1.7 is an extensible programme for visualization and analysis of molecular structure and related data including density maps, supramolecular associations, sequence alignments, docking results, routes and conformational ensembles. One of the best features is the structural editing job. It can minimize the energy of molecules providing them high stability. It is also useful in removing the various other ligand present in the molecule retrieved from the site and edited and saved for further use.

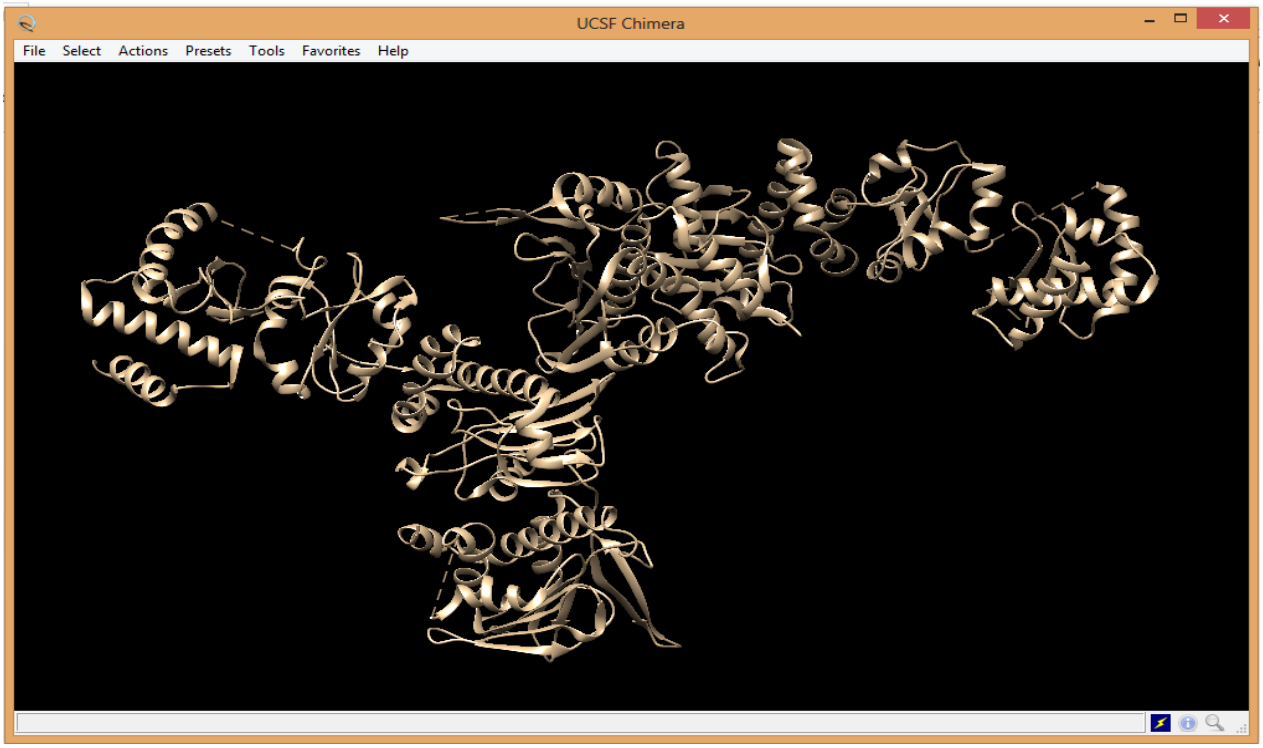


Figure 8. Energy minimization of Hsp90 in Chimera 1.7.

3.3.5 Geometry Optimization of Hsp90 molecule using ArgusLab 4.0:

Argus Lab is one of the important software which is used for the geometry optimization of the Protein molecule which we need to dock. This is done because when we dock with the ligand molecules it gives a perfect result without any errors. It optimize the geometry of the molecule for better orientation.

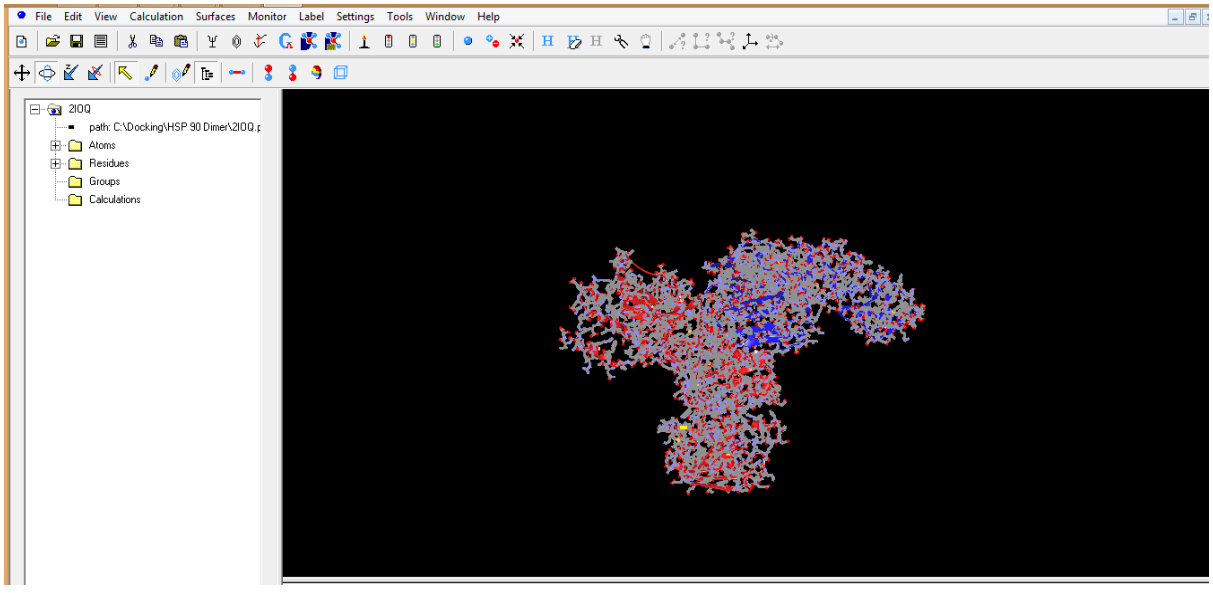


Figure 9. Geometry Optimization of Hsp90 using ArgusLab 4.0.

3.3.6 Prediction of active site of target protein Using CASTp:

Active sites of Hsp90 was predicted using CASTp. We open http://sts-fw.bioengr.uic.edu/castp/calculation.php in our taskbar. Active sites prediction of protein is important for our docking study. For these predictions we used online server CASTp. Active sites were analysed and found to contain ALA61, GLU67, CYS73, GLU78, LEU81, ALA124, THR147 residues.

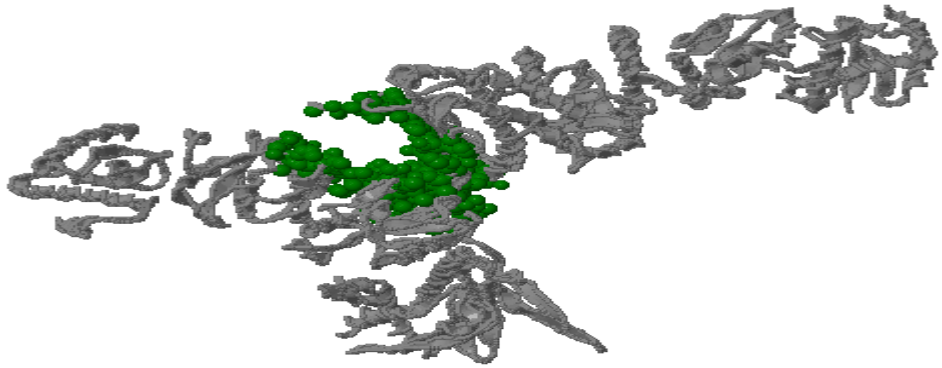


Figure 10. CASTp visualization of Hsp90 Active site amino acid residue ALA61.

3.3.7 Collection of SDF files of Hsp90 Inhibitors molecules from PubChem:

PubChem is a database of chemical structures of small organic molecules and contain information of their biological activity, origin and related literatures. It is executed and updated by NCBI and is freely available. Millions of compound structures and data seta can be freely downloaded in .sdf format or chemical (CID) format.

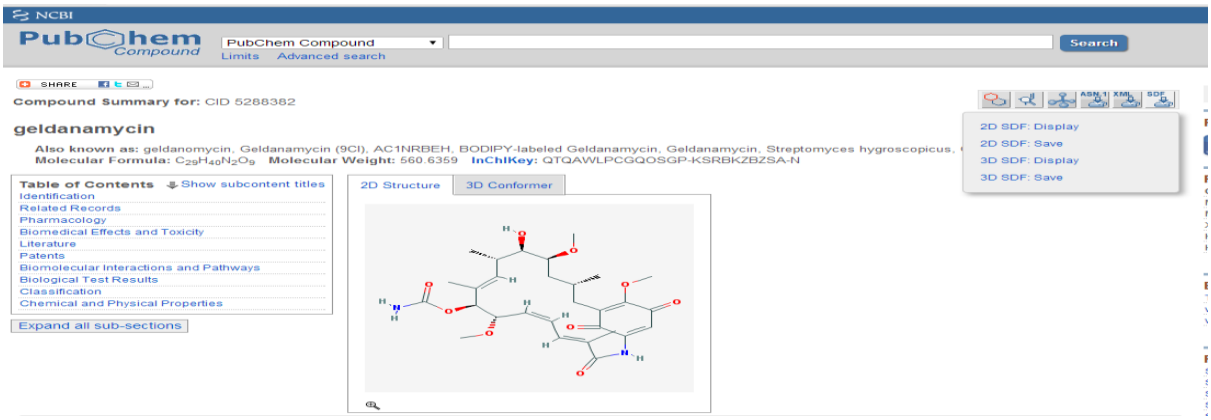


Figure 11. Structure visualization of Geldanamycin using PubChem.

3.3.8 Inhibitor PDB file convertion in OpenBabel:

Open Babel 2.3.1 is a chemical toolkit designed to interpret the various language of chemical data. It allows searching, converting, and analysing chemical data. It supports Cheminformatics, molecular modelling, and bioinformatics. It covert chemical data from one file format to another.

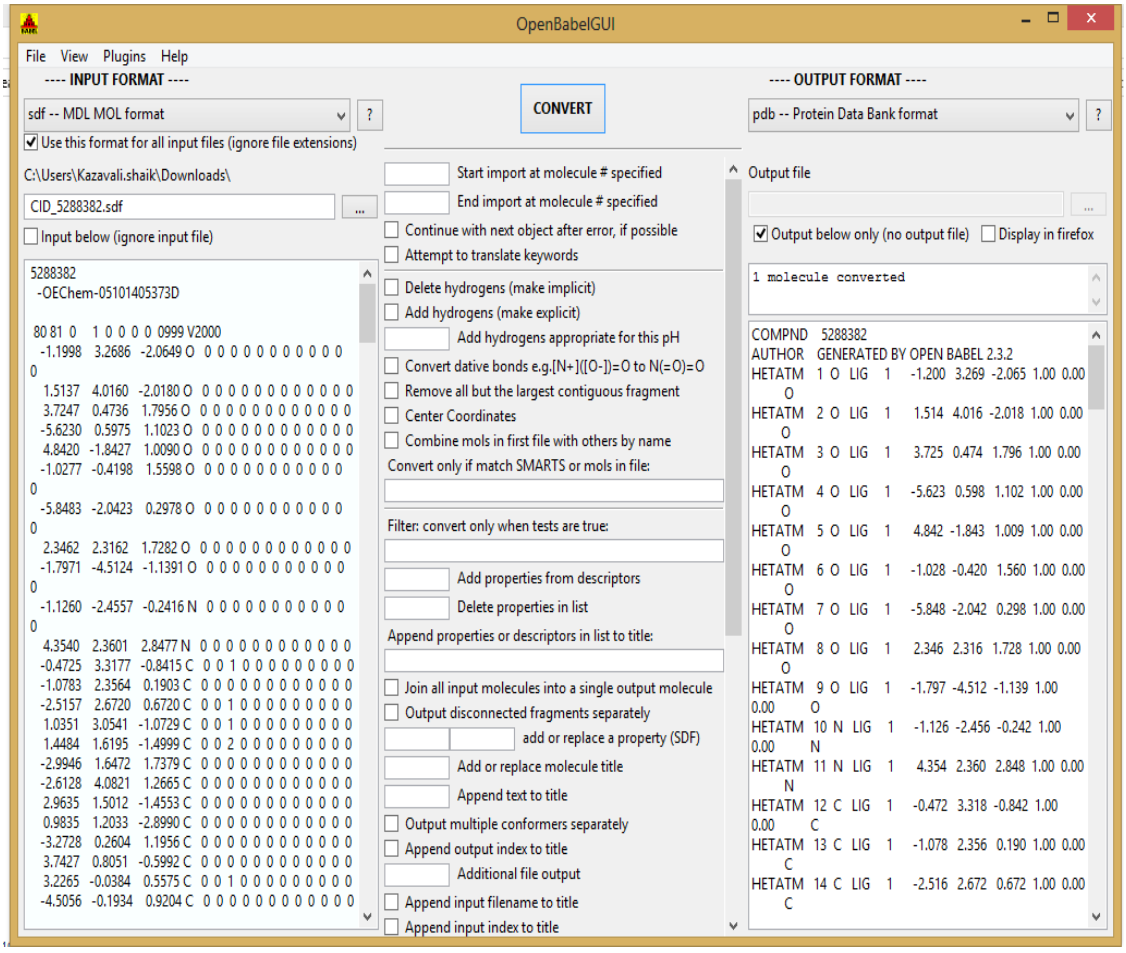


Figure 12. Visualized image of OpenBabel.

3.3.9 Use of Auto Dock 4.0 for Binding study:

Auto Dock 4.0 is an Interactive Molecular graphics program developed by The Scripps Research Institute for estimating docking calculations and displaying docking modes of pairs of protein and ligand molecules. Auto Dock is used as the docking tool which calculates intermolecular "energies" by adding up all intermolecular interactions, adding polar hydrogens and also the kollmann charges to the protein molecule (e.g. van der Waals, electrostatic) that occur between a ligand and protein target.

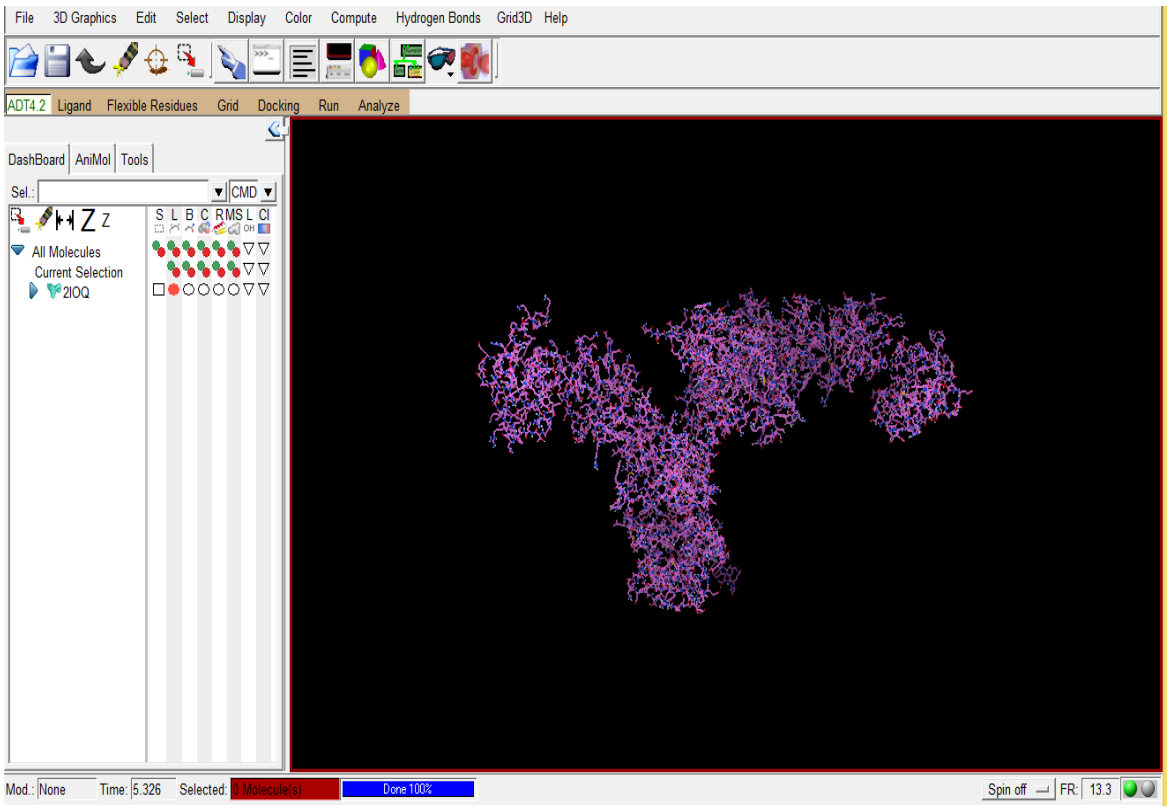


Figure 13. Visualization of Hsp90 protein in Auto Dock 4.0.

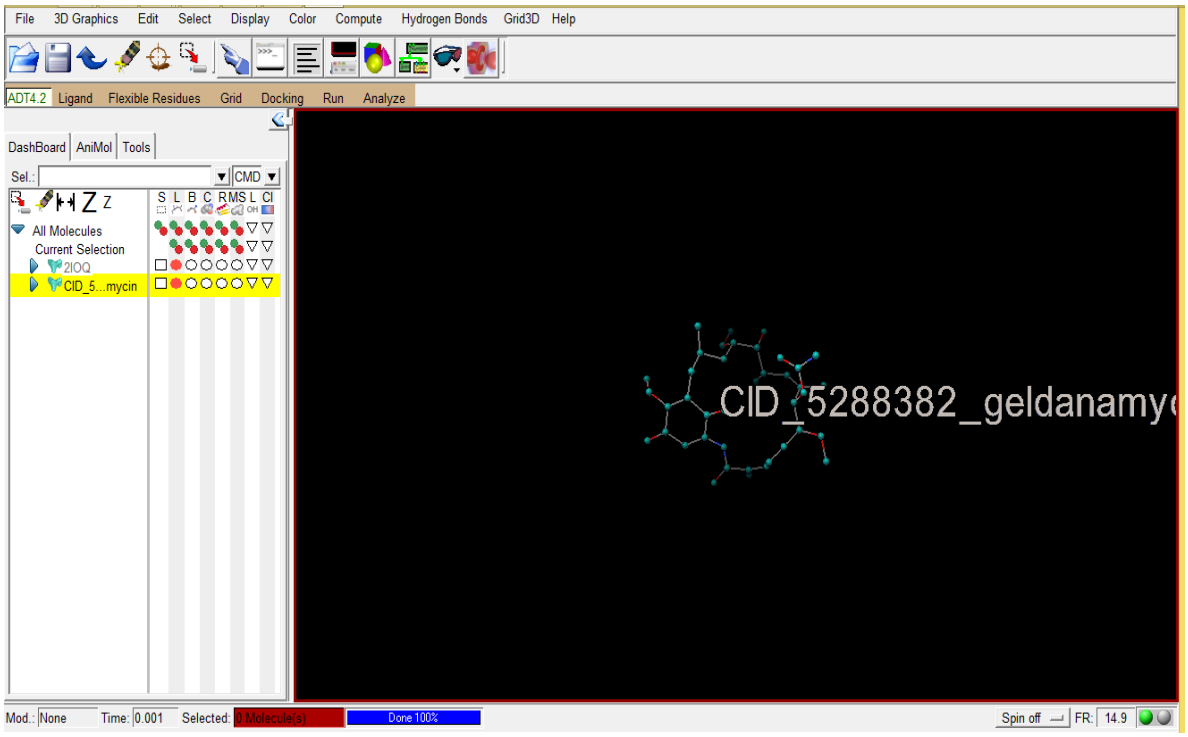


Figure 14. Visualization of Hsp90 inhibitor in Auto Dock 4.0.

3.3.10 Molecular Docking Using Auto DockVina:

Autodock Vina altogether enhances the normal exactness of the coupling mode expectations contrasted with Autodock 4, in light of different tests as per The Scripps Research Institute the preparation set utilized as a part of Autodock 4 advancement. Also and freely, Autodock Vina has been tried against a virtual screening benchmark called the Directory of Useful Decoys.

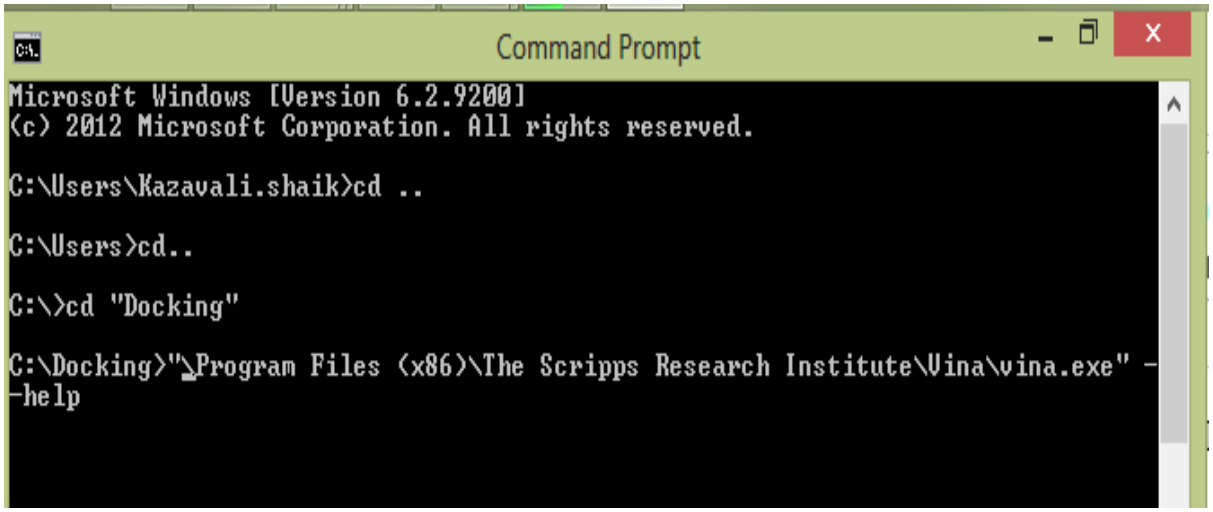


Figure 15. cmb file obtained in the first step of AutoDock vina docking.

- Create a conf.txt file giving the information of configuration for the Docking process.

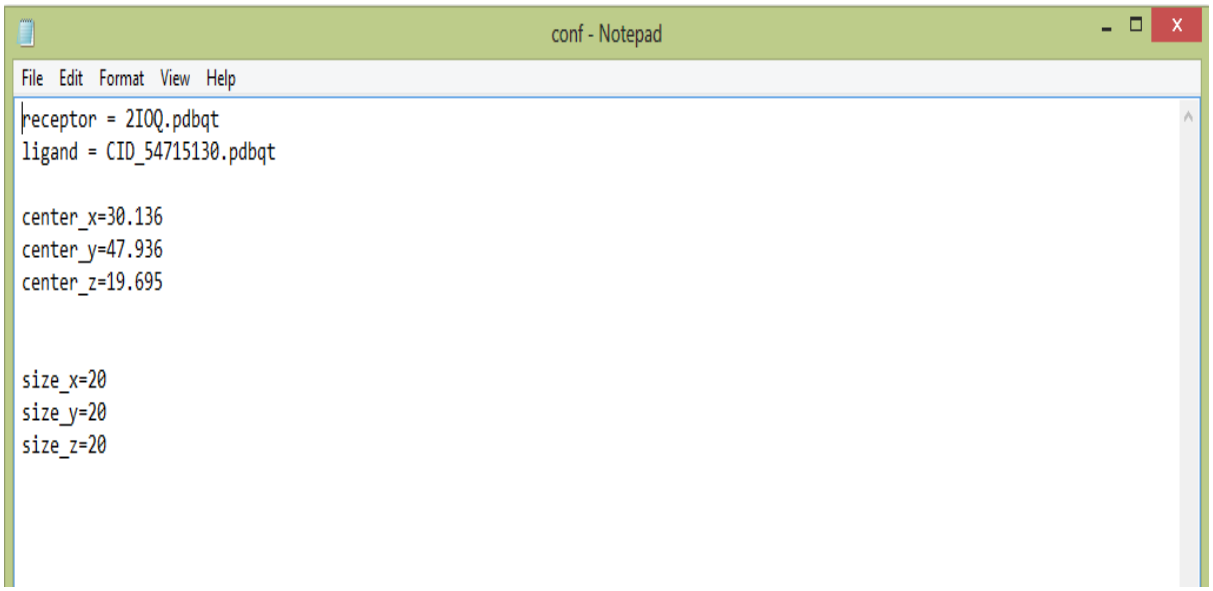


Figure 16. Coordinates of Hsp90 protein active site.

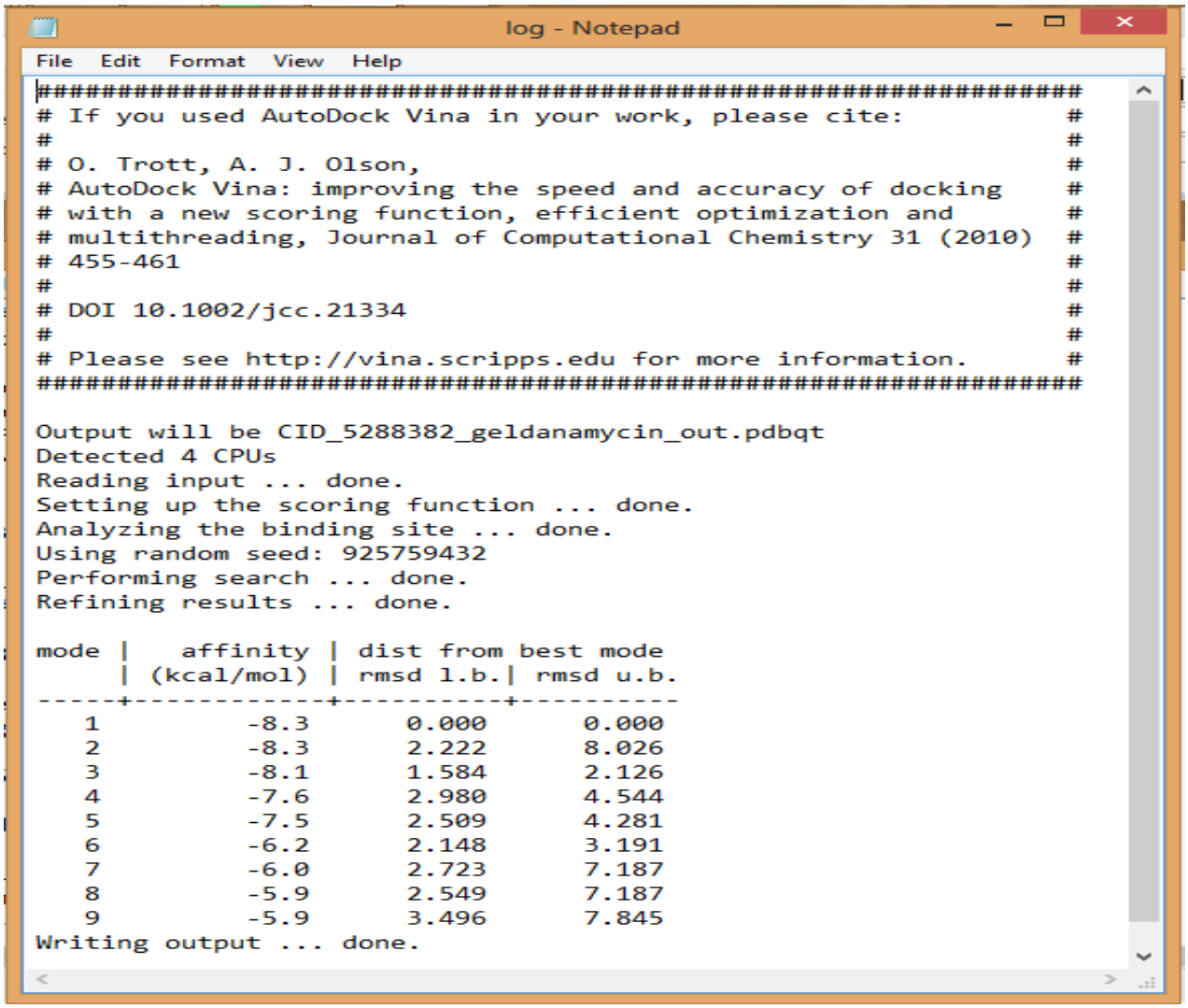


Figure 17. Output result showing binding energy obtained on docking.

3.3.11 Design of analogues of Geldanamycin and Novobiocin using ChemBioDraw13.0:

ChemBioDraw Ultra 13.0 is a tool for drawing chemical structures, adding or deleting functional group or atoms, queries and reactions. Assigning stereochemistry, charge, valence, radicals and isotopes to each atom can be done and moreover single, double, triple bonds and aromatic forms can also be created. ChemBioDraw Ultra 13.0 provides a wide range of Cheminformatics tools supporting molecule manipulation and processing, SMILES and SD file conversion, 20 normalization of molecules, and creation of tautomers, molecule disintegration,

calculation of several molecular properties needed in QSAR, molecular modelling and drug design.

- ChemBioDraw window was opened.
- The ligand .pdb format was retrieved.
- Addition, deletion of functional group changes were made keeping in mind to increase solubility and binging affinity.
- The new molecules were saved in .pdb format.

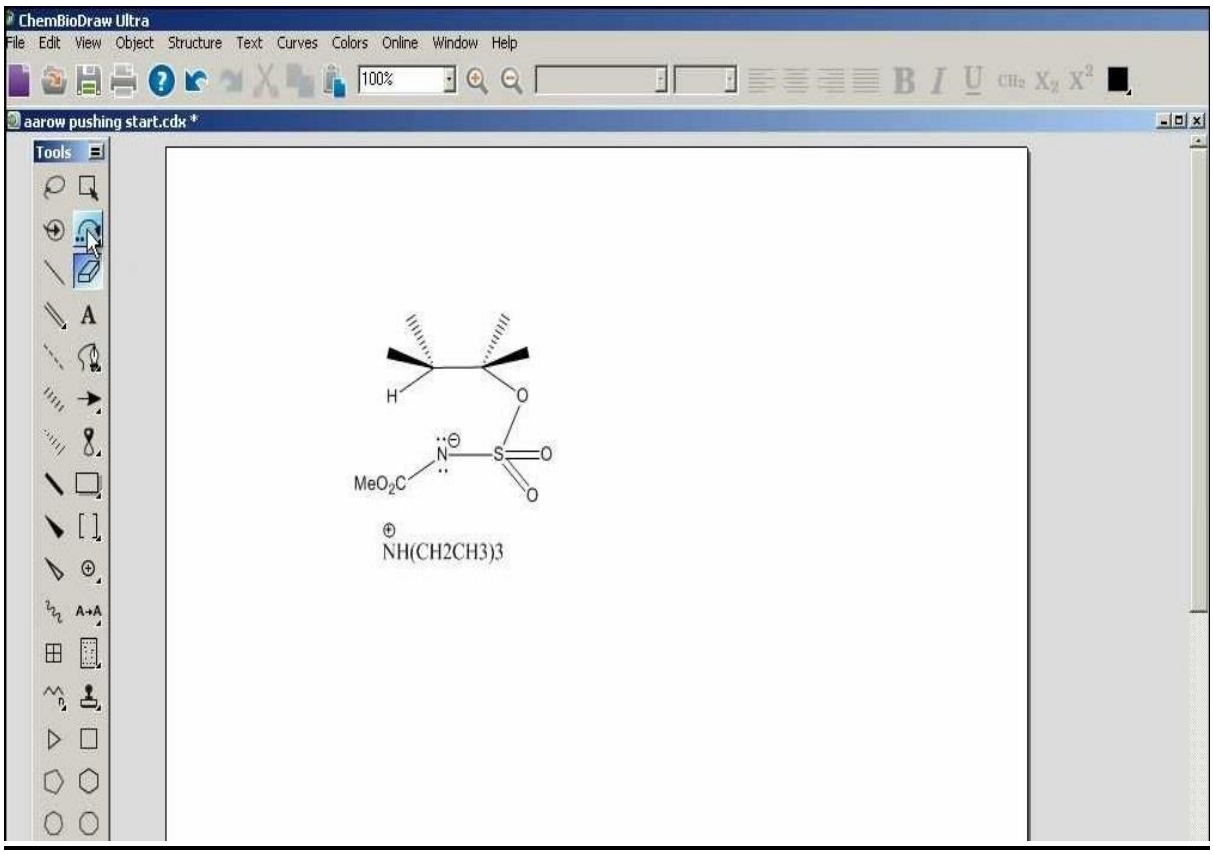


Figure 18. Visualization of analogue using ChemBioDraw13.0.

3.3.12 Docking of Hsp90 with novel Geldanamycin, Novobiocin analogues:

Autodock Vina altogether enhances the normal exactness of the coupling mode expectations contrasted with Autodock 4, in light of different tests as per The Scripps Research Institute the preparation set utilized as a part of Autodock 4 advancement. Also and freely, Autodock Vina has been tried against a virtual screening benchmark called the Directory of Useful Decoys. Docking is done using the same old soft wares given above.

Chapter 4

RESULTS AND DISCUSSION

4.1 Docking results of Hsp90 –ATP molecule:

First the Hsp90 docked with ATP molecule at various binding sites on N-terminal separately. First the Hsp90 N-terminal domain was used, and then followed the C-terminal the docking results are summarized in Table 1 .The findings of the results are solely based on the docking energy value and the interaction at the binding sites. The more negative the value, the more stable the complex is and more binding affinity. According to the energy funnel theory less energy depicts highly stable conformation. Hence more energy would be needed to break the complex that means high dissociation energy.

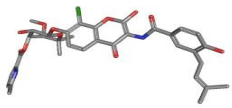
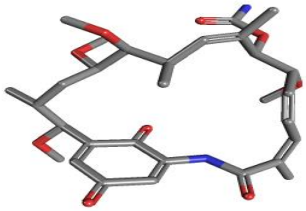
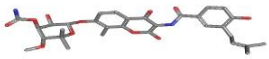
Table 1. Hsp90 with ATP molecule at different active sites.

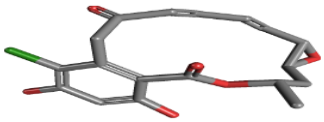
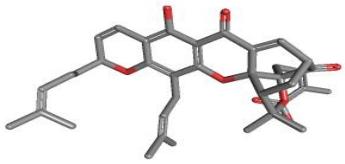
Protein	Ligand	Active Sites amino acid residue	Binding Affinity[Kcal/mol]
Hsp90	ATP	Active site amino acid residue ALA61	**-7.3**
		Active site amino acid residue GLU67	**-7.1**
		Active site amino acid residue CYS73	**-7.3**
		Active site amino acid residue ASP74	**-7.1**
		Active site amino acid residue GLU78	**-7.1**
		Active site amino acid residue LEU81	**-7.2**
		Active site amino acid residue ALA124	**-7.4**
		Active site amino acid residue THR147	**-7.5**

4.2 Docking of Hsp90 with inhibitor:

All the selected ligands were docked with Hsp90 and the results are shown in Table below.

Table 2. Results obtained on docking Hsp90 with available inhibitor [most affective].

Protein	Inhibitor	Binding Affinity[Kcal/mol]
Hsp90	 Clorobiocin	-6.8 [Active site LEU81]
	 Herbimycin	-6.4 [Active site CYS73]
	 Novobiocin	-8.3 [Active site LUE81]
	 Geldanamycin	-8.4 [Active site GLU61]

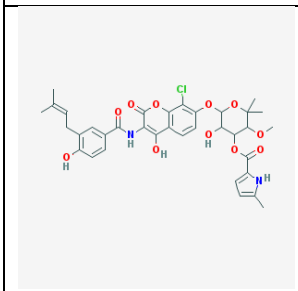
Hsp90	 Radicicol	-6.9 [Active sites ALA61,ALA124]
	 (-)-Gambogic Acid	-6.8 [Active sites ALA61,LEU81]

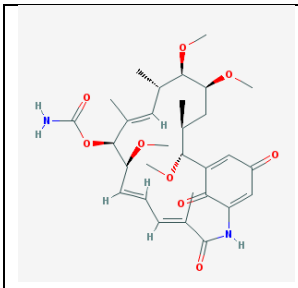
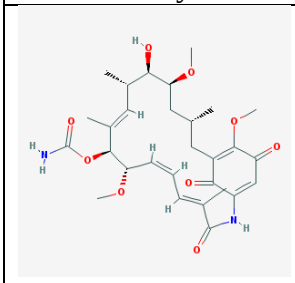
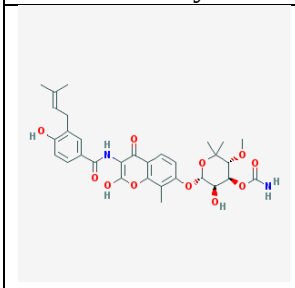
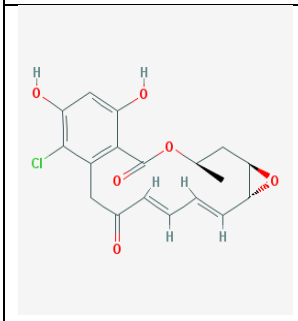
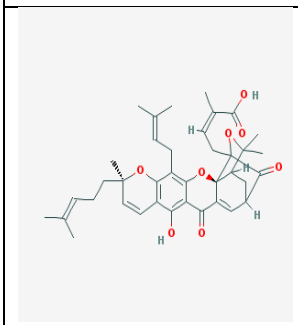
The docking of ligands was carefully observed and their interaction and orientations were also monitored. Result in Table 2 showed that Geldanamycin has the highest binding affinity (-8.4 Kcal/mol), followed by Novobiocin (-8.3 Kcal/mol).

5.3 Inhibition of Hsp90 at N-terminal and C-terminal domain:

All the selected Hsp90 N-terminal and C-terminal domain were docked with respective inhibitors for better inhibition and the results are shown in Tables 3 below.

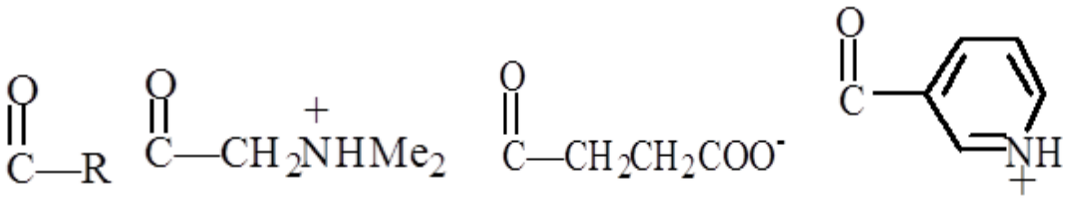
Table 3. Docking of Hsp90 with Hsp90 inhibitor at various active site.

Binding Affinity with Hsp90 [Kcal/mol]							
Inhibitor	**Active site-1**	**Active site-2**	**Active site-3**	**Active site-4**	**Active site-5**	**Active site-6**	**Active site-7**
	ALA61	**GLU67**	**CYS73**	**GLU78**	**LEU81**	**ALA124**	**THR147**
	N-terminal		**C-terminal**			**N-terminal**	
 Clorobiocin	**-5.7**	**-5.4**	**-6.7**	**-5.8**	**-6.8**	**-6.7**	**-5.6**

 Herbimycin	**-6.1**	**-5.1**	**-6.4**	**-6.3**	**-6.1**	**-6.4**	**-6.2**
 Geldanamycin	**-7.8**	**-8.4**	**-5.8**	**-5.1**	**-6.3**	**-7.6**	**-8.3**
 Novobiocin	**-5.6**	**-5.6**	**-7.8**	**-7.2**	**-8.3**	**-6.2**	**-6.2**
 Radicicol	**-6.9**	**-6.2**	**-6.1**	**-4.8**	**-5.8**	**-6.9**	**-6.8**
 (-)-Gambogic Acid	**-6.8**	**-6.3**	**-6.4**	**-5.8**	**-6.8**	**-5.8**	**-6.5**

4.4 Docking of Hsp90 with novel Geldanamycin, Novobiocin analogues

All the ligands except Clorobiocin, Herbimycin, Radicicol, and Gambogic acid showed high binding affinity than the ATP molecule, which was kept as control. Hence these ligands having high affinity than ATP can be used as Hsp90 C, N-terminal domain inhibitors. Now, the result showed Novobiocin, Geldanamycin are potent inhibitor that got matched with the literature studies. According to the research works on Novobiocin, Geldanamycin stated that it couldn't enter into the clinical trials because of its poor solubility and toxicity. The reason may be the solubility. As we know Animal cells are more aqueous in nature, hence water soluble substances easily soluble in cytosol and water insoluble form precipitates. And any extra thing in the cytosol leads to cell toxicity. This is why all the research works are going on to modify Novobiocin, Geldanamycin structures to increase its solubility. This thought inspired us to work on the modification of Novobiocin, Geldanamycin structures. With the help of ChemBioDraw Ultra 13.0, analogues were made keeping in mind that it will increase solubility. Hence the functional groups that enhance solubility and biding affinity are following:



these functional groups also enhance electronegativity that will help in forming hydrogen bonds. Hence at different position the changes were made randomly with the functional groups

Table 4. Docking results of Geldanamycin Analogues.

Protein	Inhibitor [N-terminal]	Analogues	Binding Affinity[Kcal/mol]
Hsp90	Geldanamycin	GD A1	-8.3
		GD A2	-7.4
		GD A3	-8.8
		GD A4	-7.8

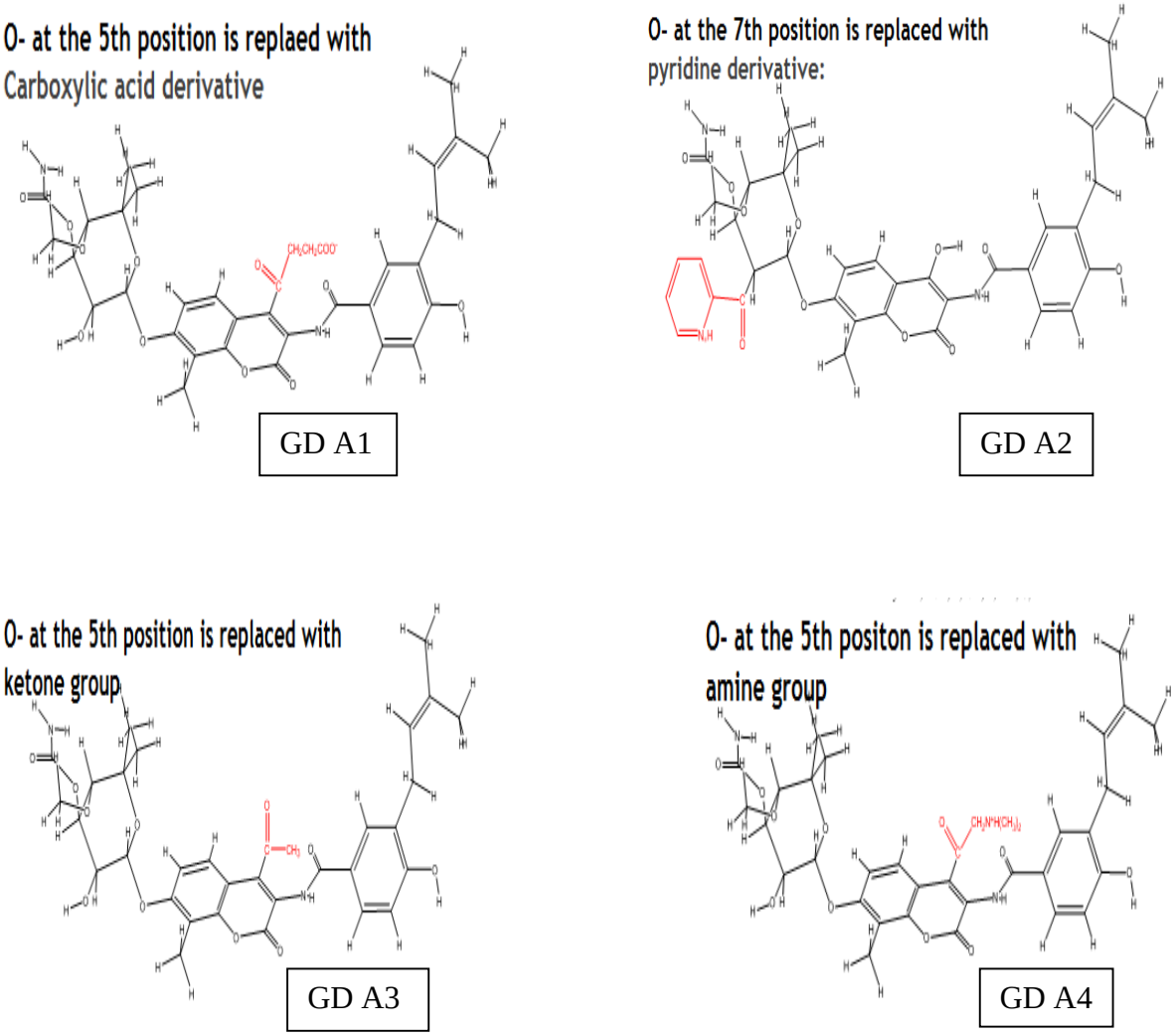
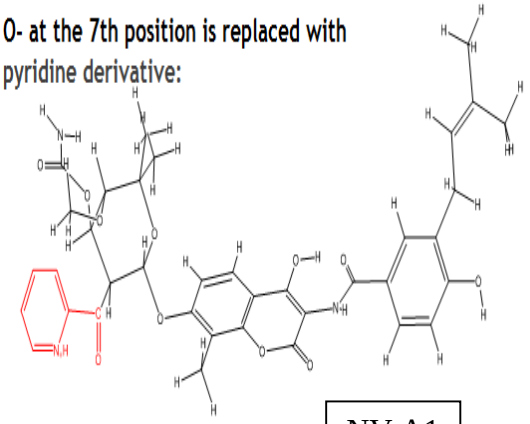


Figure 19. GD A1, A2, A3, A4 Geldanamycin Analogues prepared using ChemBiodraw13.0.

Table 5. Docking results of Novobiocin analogues.

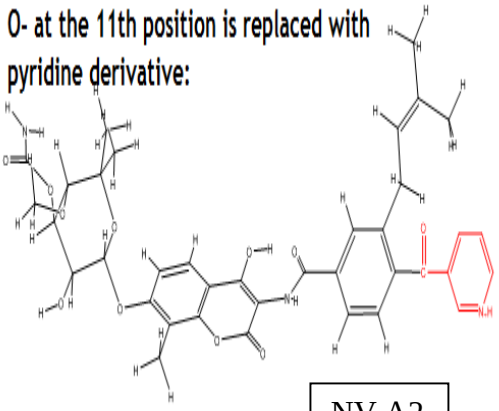
Protein	Inhibitor	Analogues	Binding Affinity[Kcal/mol]
Hsp90	Novobiocin Analogues	NV A1	-7.8
		NV A2	-7.6
		NV A3	-7.9
		NV A4	-8.0
		NV A5	-8.3
		NV A6	-8.1
		NV A7	-8.2
		NV A8	-7.9

O- at the 7th position is replaced with pyridine derivative:



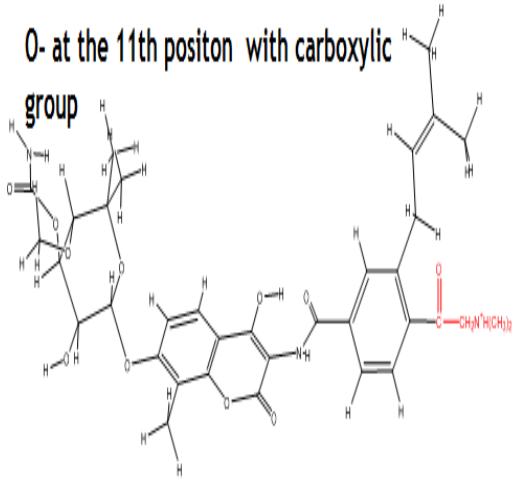
NV A1

O- at the 11th position is replaced with pyridine derivative:



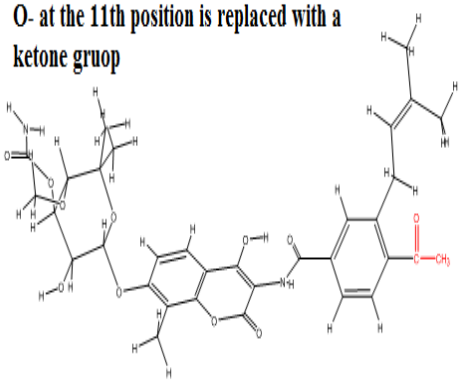
NV A2

O- at the 11th positon with carboxylic group



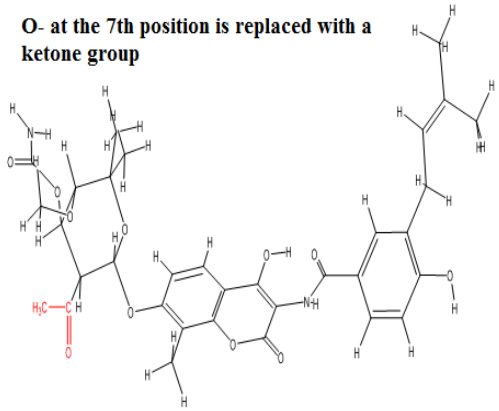
NV A3

O- at the 11th position is replaced with a ketone gruop



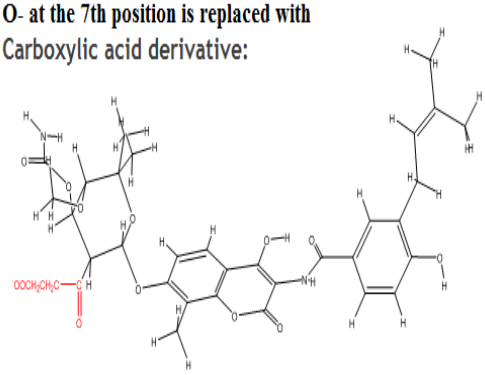
NV A4

O- at the 7th position is replaced with a ketone group

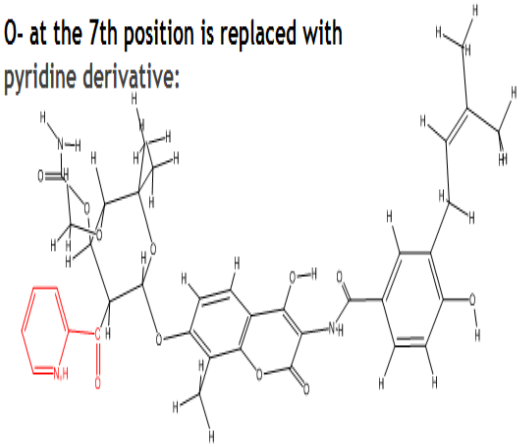


NV A5

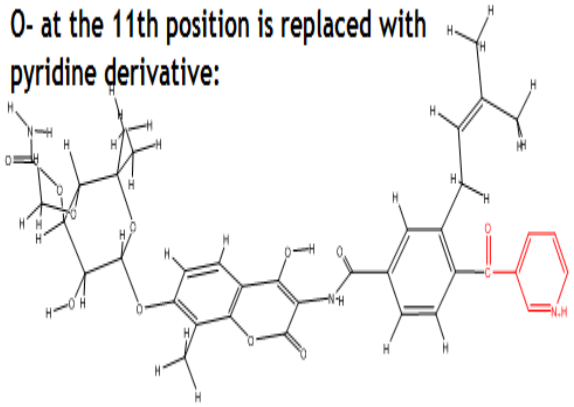
O- at the 7th position is replaced with Carboxylic acid derivative:



NV A6



NV A7

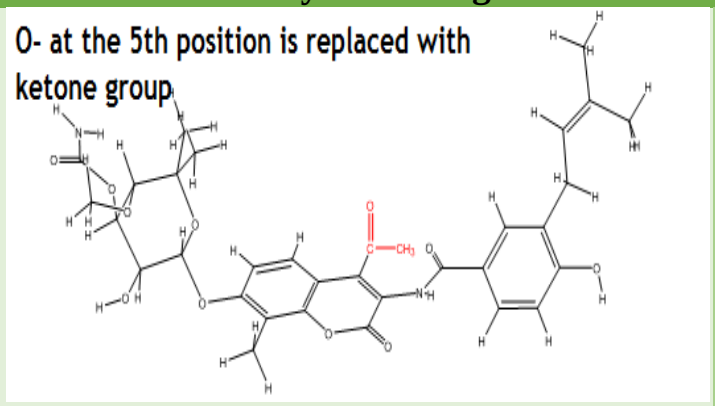


NV A8

Figure 20. NV A1, A2, A3, A4, A5, A6, A7, A8 Novobiocin Analogues made using ChemBioDraw13.0.

4.5 Final results:

From the Molecular docking results obtained we got to a conclusion that Analogue-3 of Geldanamycin showing the highest binding energy of -8.8 at N-terminal binding site residue GLU.

	Geldanamycin Analogues 3
Structure of Inhibitor	
Binding Affinity[Kcal/mol] with Hsp90	-8.8
Active Site amino acid Residue	GLU78

Chapter 5
CONCLUSION

5.1 Conclusion

Hsp90 inhibitors for the treatment of neurodegenerative disorders have been growing exponentially since identification of Geldanamycin, Redicicol as the Hsp90 inhibitor in 1994. For the last 25 years, several kinds of Hsp90 inhibitors have been identified, each with unique mechanisms of inhibition and each displaying somewhat different biological effects. Several of these inhibitors have been clinically evaluated for a variety of different neurodegenerative disorders, unfortunately, the results of these trials has somewhat been disappointing. The results from these trials emphasize the need of thoroughly understanding the biology of the target, better understanding how different kinds of inhibitors affect the molecular chaperone, and to determine how cellular environments affect drug efficacy. Several strategies exist that may help circumvent the known pitfalls resulting from Hsp90 inhibition. In summary, Hsp90 is still an attractive therapeutic target, but finding new strategies for inhibition are necessary to overcome the clinical liabilities observed for inhibitors.

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